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Separation and Quantitation of Red Pepper Major Heat Principles by Reverse-Phase High-Pressure Liquid Chromatography

Patrick G. Hoffman,* Mary C. Lego, and William G. Galetto

A rapid, reverse-phase high pressure liquid chromatographic method is presented that will identify and quantitate the major heat principles (capsaicinoids) in red pepper products. The capsaicinoids are extracted from the ground spice with ethanol, separated by high-pressure liquid chromatography, and detected by ultraviolet absorption at 280 nm. The concentration of the individual capsaicinoids is determined relative to that of a commercially available external standard, *N*-vanillyl-*n*-nonamide (*N*-[(4-hydroxy-3-methoxyphenyl)methyl]-*n*-nonamide). The data obtained by this method can be mathematically transformed into a sensory value.

The compound primarily responsible for the pungency of the capsicums is capsaicin (*N*-[(4-hydroxy-3-methoxyphenyl)methyl]-8-methyl-6-nonenamide). Other structurally similar heat contributors in red pepper include dihydrocapsaicin (*N*-[(4-hydroxy-3-methoxyphenyl)methyl]-8-methylnonanamide), and, to a lesser extent, norcapsaicin (*N*-[(4-hydroxy-3-methoxyphenyl)methyl]-7-methyl-5-octenamide), nordihydrocapsaicin (*N*-[(4-hydroxy-3-methoxyphenyl)methyl]-7-methyloctanamide), homocapsaicin (*N*-[(4-hydroxy-3-methoxyphenyl)methyl]-9-methyl-7-decenamide), and homodihydrocapsaicin (*N*-[(4-hydroxy-3-methoxyphenyl)methyl]-9-methyldecanamide). Additional related capsaicinoids have been identified as trace constituents of these products (Jurenitsch et al., 1979a,b).

A review of the literature reveals a variety of wet chemical and instrumental methods to identify and quantitate these compounds in natural products. Thin-layer chromatography (TLC), TLC coupled with ultraviolet (UV) absorbance detection at 280 nm, gas chromatography (GC), GC coupled with mass spectrometry (MS), the combination of high-pressure liquid chromatography (HPLC), GC, and MS as well as mass fragmentation with quantitation by computer analysis (Todd and Perun, 1961; Hartman, 1970; Masada et al., 1971; Tirimanna, 1972; Govindarajan and Ananthakrishna, 1974; Todd et al., 1975; DiCecco, 1976; Lee et al., 1976; Polesello and Pizzocaro, 1976; Kosma-Kovacs et al., 1977; Palacio, 1977; Pankar and Magar, 1977; Todd et al., 1977; Pankar and Magar, 1978; DiCecco, 1979; Heresch and Jurenitsch, 1979; Iwai et al., 1979; Bajaj, 1980; Suzuki et al., 1980; Rajpoot and Govindarajan, 1981) are all significant efforts toward this endeavor. Most of these procedures require derivatization of the capsaicinoids prior to analysis and/or complex instrumentation, involved preparation time, and specialized knowledge. The most effective methods quantitate the individual capsaicinoids in order to account for the different heat contributions.

Research and Development Laboratories (P.G.H. and M.C.L.) and McCormick/Stange Flavor Division (W.G.G.), McCormick and Company, Inc., Hunt Valley, Maryland 21031.

Table I. Sensory Values of the Individual Capsaicinoids ($\times 10^6$)[a]

	sensory values
nordihydrocapsaicin	9.3 ± 0.4
capsaicin	16.1 ± 0.6
dihydrocapsaicin	16.1 ± 0.6
homocapsaicin	6.9 ± 0.5
homodihydrocapsaicin	8.1 ± 0.7
N-vanillyl-*n*-nonamide	9.2 ± 0.5

[a] Todd et al. (1977).

One objective method that separates and quantitates the capsaicinoids is presented by Todd et al. (1977). This method correlates a sophisticated sensory analysis with an equally complex GC separation and quantitation of the individual capsaicinoids. The capsaicinoids—nordihyrocapsaicin, capsaicin, dihydrocapsaicin, homocapsaicin, and homodihydrocapsaicin—are synthesized and the pungencies determined for each compound by a series of carefully controlled, sensory triangle tests. The threshold pungencies of the heat compounds, as determined by this sensory method, are reported in Table I.

High-pressure liquid chromatographic methods, however, have a strong advantage over GC approaches in that derivatization is not required in order to quantitate the individual capsaicinoids (Sticher et al., 1978; Johnson et al., 1979; Jurenitsch et al., 1979a; Woodbury, 1980). Advances in both the instrumentation and methodology allow routine application of HPLC analysis.

A reproducible method that uses common reagents to separate, identify, and quantitate individual capsaicinoids on a routine basis was the objective of our research. Like Sticher et al. (1978), Jurentisch et al. (1979a), and Woodbury (1980), we chose HPLC as the analytical tool. As noted earlier, HPLC methods do not require derivatization and, therefore, are simple and direct.

Our research involved the synthesis, isolation, separation, and quantitation of the capsaicinoids.

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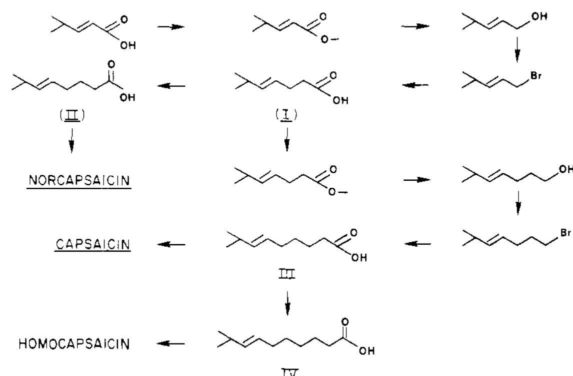
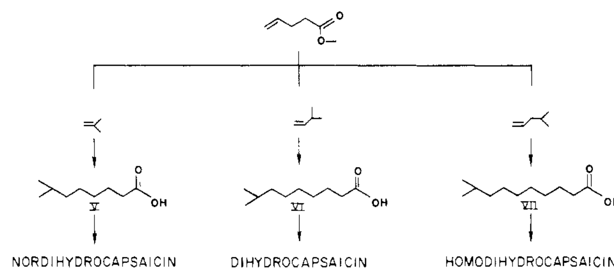
Scheme I. Synthesis of Unsaturated Capsaicinoid Fatty Acids



Scheme II. Synthesis of Saturated Capsaicinoid Fatty Acids



MATERIALS AND METHODS

Synthetic Capsaicinoids. The synthetic compounds necessary for the identification and quantitation of the individual capsaicinoids were prepared by innovative chain extensions or carbon-carbon coupling sequences.

The unsaturated homologous series—norcapsaicin, capsaicin, and homocapsaicin—was synthesized by the appropriate application of the malonic ester two-carbon extension and the Arndt-Eistert one-carbon extension (Scheme I). Thus, the methyl ester (Clinton and Laskowski, 1948) of *trans*-4-methyl-2-pentenoic acid was reduced with sodium bis(2-methoxyethoxy)aluminum hydride to the alcohol (Technical Information Bulletin, 1974). This product was converted to the bromide with PBr_3 (Wiley et al., 1964). A malonic ester transformation (Sandler and Karo, 1968a) gave 6-methyl-4-heptenoic acid (compound I). This acid was increased in length by one carbon with the standard Arndt-Eistert synthetic rearrangement (Sandler and Karo, 1968b); formation of the diazoketone from the acid chloride and diazomethane, followed by rearrangement with $Na_2S_2O_3$ and Ag_2O, produced the iso fatty acid unsaturated portion of norcapsaicin, 7-methyl-5-octenoic acid(compound II).

For the capsaicin precursor, the acid (compound I) was reintroduced into the two-carbon extension sequence: methyl esterification, reduction to the alcohol, conversion to the bromide, and transformation with the malonic ester synthesis. The resulting compound was 8-methyl-6-nonenoic acid (compound III). The homocapsaicin fatty acid, 9-methyl-7-decenoic acid (compound IV), was synthesized from the acid (compound III) by the Arndt-Eistert sequence, one-carbon extension, previously described. The acid chlorides of II, III, and IV, formed by the action of $SOCl_2$ on the appropriate acid, were each reacted in ether with a 2-fold excess of vanillylamine freed from its hydrochloride salt. After the standard workup, major contaminants were removed by column chromatography on silica gel grade 950, 60–200 mesh (W. R. Grace), eluting the capsaicinoids with petroleum ether containing a slowly increasing concentration of ether. Each of the eluted compounds was further purified by TLC and/or HPLC. The TLC separations were accomplished on 200-μm silica gel GF plates (Analtech) and developed with 3:1 ether–petroleum ether. Preparative HPLC work was done on a Prep 500 (Waters Associates) utilizing one C_{18} column and a solvent system of 50:50 CH_3CN–H_2O (1% HOAC). The flow rate was 0.25 L/min. The capsaicinoids, freed from contaminating byproducts, were each recrystallized from hexane several times.

The saturated homologous series—nordihydrocapsaicin, dihydrocapsaicin, and homodihydrocapsaicin—was synthesized by the unique application of an organoborane alkyl coupling reaction suggested by Brown (1972, 1975). This involved forming a mixed trialkylborane from the pertinent primary olefins. With the appropriate choice and concentration of olefins, the coupling to the desired product with $AgNO_3$ was favored and this compound was separated easily from the other expected products (Scheme II). Thus, the methyl ester (Clinton and Laskowski, 1948) of 4-pentenoic acid and a 3-fold excess of isobutylene or 3-methyl-1-butene, or 4-methyl-1-pentene were reacted with BH_3–THF. The coupling of the alkyl groups was accomplished in situ with KOH in MeOH followed by $AgNO_3$. The esters were hydrolyzed with additional KOH and the mixture of products was isolated after acidification. A simple distillation separated the lower boiling symmetrical hydrocarbon from the desired iso fatty acid and the high-boiling symmetrical diacid remained in the distillation flask. The isolated iso fatty acids were 7-methyloctanoic acid (compound V), 8-methylnonanoic acid (compound VI) and 9-methyldecanoic acid (compound VII).

Each of the acid chlorides formed by the reaction of $SOCl_2$ on these acids was reacted with vanillylamine and purified as previously described for the unsaturated series, thereby producing the expected capsaicinoids.

Commercially available *N*-vanillyl-*n*-nonamide (*N*-[(4-hydroxy-3-methoxyphenyl)methyl]-*n*-nonamide) was scrupulously purified by repeated recrystallization from hexane for use as the external standard. The identification of the capsaicinoids was confirmed by MS as well as NMR and UV where the availability of pure sample was adequate. The ^1H NMR spectra were recorded on a Varian EM-360 spectrometer. The chemical shifts are given in parts per million (ppm) relative to internal tetramethylsilane. Mass spectra were taken on a Du Pont 21-490 or a Du Pont DP-102 mass spectrometer at 70 eV. The ultraviolet spectra were obtained on a Beckman DB-GT grating spectrophotometer.

N-Vanillyl-n-nonamide (ICN Laboratories). $C_{17}H_{27}NO_3$; M_r 293; ^1H NMR ($CDCl_3$) δ 6.77 (m, 3), 5.81 (br m, 2), 4.35 (d, 2), 3.84 (s, 3), 2.20 (t, 2), 1.95–1.1 (brs, 12), 0.76 (t, 3); $UV_{\lambda_{max}}^{EtOH}$ 281 nm (ϵ 3230), 229 (7770); MS (70 eV) *m/e* (rel intensity) 294 (22), 293 (56), 195 (27), 152 (13), 151 (13), 138 (15), 137 (100), 136 (62), 43 (8), 41 (9).

Norcapsaicin. $C_{17}H_{25}NO_3$; M_r 291; ^1H NMR ($CDCl_3$) δ 6.79 (m, 3), 6.28 (brs, 1), 6.21 (brs, 1), 5.39 (m, 2), 4.34 (d, 2), 3.84 (s, 3), 2.49–1.42 (m, 7), 0.94 (d, 6); MS (70 eV) *m/e* (rel intensity) 292 (12), 291 (60), 195 (22), 152 (12), 151 (14), 138 (12), 137 (100), 122 (7), 55 (8), 41 (9).

Nordihydrocapsaicin. $C_{17}H_{27}NO_3$; M_r 293; ^1H NMR ($CDCl_3$) δ 6.85 (m, 3), 6.12 (s, 1), 6.08 (brs, 1), 4.35 (d, 2), 3.85 (s, 3), 2.21 (t, 2), 1.9–1.0 (brm, 9), 0.86 (d,6); $UV_{\lambda_{max}}^{EtOH}$ 280 nm (ϵ 3110), 228 (7490); MS (70 eV) *m/e* (rel intensity) 293 (25), 152 (13), 151 (12), 138 (14), 137 (100), 136 (15), 122 (14), 72 (18), 71 (15), 69 (54), 59 (27), 57 (30), 55 (21), 44 (33), 43 (27), 41 (23).

Capsaicin. $C_{18}H_{27}NO_3$; M_r 305; ^1H NMR ($CDCl_3$) δ 6.77 (m, 3), 6.07 (brm, 2), 5.31 (m, 2), 4.32 (d, 2), 3.81 (s, 3),

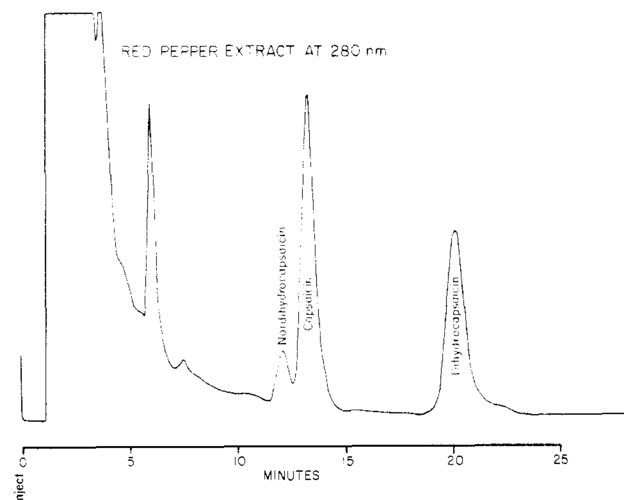


Figure 1. Separation of red pepper capsaicinoids by high-pressure liquid chromatography: column μBondapak C_{18}; sample size, 50 μL; flow rate, 1.5 mL/min.; solvent, acetonitrile–water (1% acetic acid), 40:60.

2.5–1.18 (m, 9), 0.92 (d, 6); $UV_{\lambda_{max}}^{EtOH}$ 280.5 nm (ε 2690), 230 (8130); MS (70 eV) *m/e* (rel intensity) 306 (17), 305 (71), 195 (7), 168 (6), 152 (13), 151 (7), 138 (15), 137 (100), 122 (9), 55 (12).

Dihydrocapsaicin. $C_{18}H_{29}NO_3$; M_r 307; ^1H NMR ($CDCl_3$) δ 6.71 (m, 3), 5.63 (brm, 2), 4.29 (d, 2), 3.84 (s, 3), 2.13 (t, 2), 1.85–1.0 (brm, 11), 0.82 (d, 6). $UV_{\lambda_{max}}^{EtOH}$ 281 nm (ε 2630), 228 (6390); MS (70 eV) *m/e* (rel intensity) 308 (18), 307 (100), 306 (7), 195 (28), 194 (43), 152 (10), 151 (11), 138 (12), 137 (87), 136 (6), 43 (16), 41 (12).

Homocapsaicin. $C_{19}H_{29}NO_3$; M_r 319; ^1H NMR ($CDCl_3$) δ 6.78 (m, 3), 5.87 (brm, 2), 5.33 (m, 2), 4.33 (d, 2), 3.86 (s, 3), 2.45–1.10 (brm, 11), 0.97 (d, 6). $UV_{\lambda_{max}}^{EtOH}$ 282 nm (ε 3180), 231 (7720); MS (70 eV) *m/e* (rel intensity) 320 (17), 319 (61), 195 (5), 152 (9), 138 (12), 137 (100), 122 (6), 69 (8), 55 (11), 41 (9).

Homodihydrocapsaicin. $C_{19}H_{31}NO_3$; M_r 321; MS (70 eV) *m/e* (rel intensity) 321 (32), 195 (60), 152 (20), 151 (24), 138 (18), 137 (100), 59 (22), 55 (20), 43 (33), 41 (27), 39 (9).

HPLC Separation. Separation of the capsaicinoids was accomplished on a Waters Associates ALC/GPC high-pressure liquid chromatograph with a 10-μm μ Bondapak C_{18} column and a guard column of Bondapak C_{18}/Corasil (Waters Associates) with detection at 280 nm. The isocratic mobile phase was acetonitrile–water (1% acetic acid), 40:60 v/v, controlled by a Model 660 solvent programmer (Waters Associates). The flow rate was 1.5 mL/min and the injection size was 50 μL of a solution at 20 °C from a 100-μL Hamilton LC syringe via a U6K injector (Waters Associates).

Sample Preparation. The capsaicinoids were extracted from 25.0 g of the ground red pepper in 200.0 mL of 95% ethanol by heating at 65–75 °C for 5 h. The suspended material was allowed to settle and a sample of the supernatant was transferred to a Teflon-lined screw-cap vial. The sample was brought to 20 °C. Fifty microliters of the solution was injected directly into the HPLC system and the capsaicinoids were detected at 280 nm.

RESULTS AND DISCUSSION

The capsaicinoids are individually distinguished by the fatty acid substituent. For this reason, a reverse-phase HPLC system of separation was developed.

The major capsaicinoids in the various red peppers were identified by retention time as nordihydrocapsaicin, capsaicin, and dihydrocapsaicin. These major heat principles individually contribute the most to sensory heat (Table I). The other capsaicinoids were either not detected by this method or found to be present in only trace quantities. This confirms the earlier GC (Todd et al., 1977) and LC (Sticher et al., 1978; Jurenitsch et al., 1979a) findings, which concluded that homocapsaicin and homodihydrocapsaicin were present only at low levels and norcapsaicin was not reported. The solvent system described eluted extraneous material early in the chromatogram, which allowed almost base-line resolution of the capsaicinoids (see Figure 1).

Table II. Relative Retention Times of Capsaicinoids by Reverse-Phase HPLC

capsaicinoid	relative retention time
norcapsaicin	0.67
nordihydrocapsaicin	0.92
N-vanillyl-*n*-nonamide	1.00
capsaicin	1.02
dihydrocapsaicin	1.52
homocapsaicin	1.65
homodihydrocapsaicin	2.52

Table III. Capsaicinoid Response Factors and Linear Ranges at 280 nm[a]

	response factors	linear range, μg
N-vanillyl-*n*-nonamide	1.0	0.5–372
nordihydrocapsaicin	0.98	0.5–99
capsaicin	0.89	0.6–49
dihydrocapsaicin	0.93	0.5–83

[a] Identification and quantification of the individual capsaicinoids were made by comparing the peak retention time and area relative to those of the external standard analyzed under identical HPLC conditions.

In order to facilitate identification of the individual capsaicinoids by laboratories not in possession of purified standards, a retention time for each capsaicinoid was determined relative to an external standard, *N*-vanillyl-*n*-nonamide (Table II). This material is commercially available and is structurally similar to the capsaicinoids. This elution order confirms that reported by Sticher et al. (1978) and Jurenitsch et al. (1979a), which is related to the fatty acid chain length and degree of saturation.

As is apparent from the relative retention values, the possible red pepper product adulterant, *N*-vanillyl-*n*-nonamide, would not be differentiated by this technique. However, simple isolation of the suspected material and analysis by NMR or MS would detect the presence of this compound.

In order to establish the linear range of the detector response to the capsaicinoids and *N*-vanillyl-*n*-nonamide (the external standard), six to seven dilutions of several stock solutions of each of the synthetic capsaicinoids were injected into the HPLC.

Peak areas were determined by integration (Data Module, Waters Associates). Correlation coefficients of the HPLC data and the concentrations of these four compounds were all greater than 0.99.

Although an integrator was used for this work, it was possible to quantitate the capsaicinoids by triangulation and/or peak height with a slight reduction in precision. The response factors, based on peak area, relative to the external standard (N-vanillyl-n-nonamide) and the linear ranges of the capsaicinoids are listed in Table III. Ranges examined for the capsaicinoids were limited due to the quantities of available synthetic standards. The extended

range of the commercially available *N*-vanillyl-*n*-nonamide would be suggestive of similar ranges for the chemically related natural compounds.

The following formula was used for the quantitation of each of the capsaicinoids:

$$(PA/RF)(QS/AS)/S \times 100 = \% \text{ capsaicinoid}$$

PA = area counts of the capsaicinoid peak of interest, RF = the response factor of the individual capsaicinoid, QS = the quantity of the standard (*N*-vanillyl-*n*-nonamide) injected, AS = area counts of the standard, and S = the quantity of the sample injected. The heat contribution of each component can be determined by multiplying the percentage of that constituent by the appropriate Todd value (see Table I). The total heat value of the product can then be determined by summing these individual contributions.

Several recovery experiments established the effectiveness of this procedure. Ground red peppers (25 g) were analyzed by this method to determine their natural capsaicinoid composition. The samples were found to contain 3.20 mg of nordihydrocapsaicin, 28.10 mg of capsaicin and 19.96 mg of dihydrocapsaicin, respectively. Each sample was fortified with purified synthetic compound (9.10 mg of nordihydrocapsaicin, 25.36 mg of *N*-vanillyl-*n*-nonamide, and 1.75 mg dihydrocapsaicin, respectively). *N*-vanillyl-*n*-nonamide was used because of its availability and chemical similarity to capsaicin. Extraction and quantitation were completed as previously described. Recoveries, calculated according to the suggestion of Boyer (1981), were as follows: nordihydrocapsaicin, 98.9% with a total of 12.20 mg recovered; capsaicin/*N*-vanillyl-*n*-nonamide, 100.6% with a total of 53.60 mg recovered; dihydrocapsaicin, 98.3% with a total of 21.68 mg recovered. To confirm the reproducibility of this method, a single red pepper was analyzed 5 times, producing a mean value of 0.198% capsaicinoids with a standard deviation of 0.004%.

Although this method of analysis separated pigments and other interfering compounds early in the chromatogram, sample cleanup procedures were examined in an effort to retard column degradation. These methods included selective solvent extraction (cyclohexane, liquid freon) and column cleanup on silica or alumina. All attempts to remove the pigments from the extract reduced the recovery of compounds of interest. Elution on preparative thin-layer plates was evaluated but proved to be too involved and time consuming for routine analysis. Therefore, the described direct injection of a dilute ethanol extract was selected. This prevented loss of material and applied limited levels of extraneous material to the column.

During the development of the extraction procedure, various solvents were examined in order to determine the most suitable medium for isolating the capsaicinoids. The solvents tested included acetonitrile, acetone, methylene chloride, and ethanol. Acetonitrile required a lengthy extraction time and carried with it extraneous interfering compounds. Acetone, although an excellent solvent for removing the capsaicinoids (Sankarikutty, 1978), adversely affected the resolution of the individual compounds during the HPLC analysis. Methylene chloride extracted the materials in a reasonable time but was impactical because it carried residues not totally miscible with the mobile phase. Ethanol (Sankarikutty, 1978; Woodbury, 1980) extracted greater than 98% of the capsaicinoids after 5 h (see Figure 2). This was determined by extensive reextraction of the analyzed sample, yielding 0.52 mg additional capsaicinoids as compared to the 28.76 mg obtained after 5 h. Use of this solvent permitted direct injection of the extract, eliminating possible losses that occur during filtering, concentration, or gross transfer. A representative group of 55 ground red peppers that included most commercial varieties was analyzed by using the described method. The capsaicinoid ranges of these products are reported in Table IV.

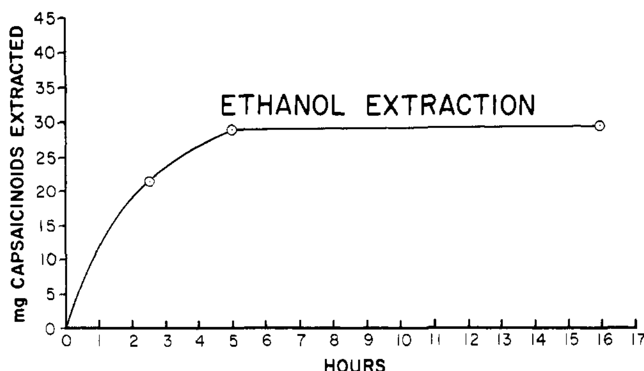


Figure 2. Ethanol extraction of red pepper capsaicinoids.

Table IV. Concentration Ranges of Major Naturally Occurring Capsaicinoids[a]

capsaicinoid	concentration range, %
nordihydrocapsaicin	0.004-0.030
capsaicin	0.047-0.277
dihydrocapsaicin	0.017-0.152

[a] The remaining capsaicinoids (norcapsaicin, homocapsaicin, and homodihydrocapsaicin) did not occur with any regularity or at quantitatable levels under these conditions.

No particular problems were encountered with the instrumentation during this research. The guard column was repacked with Bondapak C_{18}/Corasil (Waters Associates) after approximately every 50 injections and the column was washed with straight acetonitrile at the same interval. These maintenance procedures prevented column degradation. Retention time shifts were negligible throughout the study, and separation (α) values remained stable.

CONCLUSION

The methodology described is one of several acceptable techniques for objective determination of the heat level of capsicum products. The method is direct, safe, and reproducible and can be utilized in most laboratories on a routine basis without special precautions or unusual and dangerous reagents.

Registry No. Norcapsaicin, 61229-08-1; nordihydrocapsaicin, 28789-35-7; capsaicin, 404-86-4; dihydrocapsaicin, 19408-84-5; homocapsaicin, 58493-48-4; homodihydrocapsaicin, 20279-06-5.

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Vitamin E Content of Feedstuffs Determined by High-Performance Liquid Chromatographic Fluorescence

Winifred M. Cort,* Thelma S. Vicente, Edward H. Waysek, and Beverly D. Williams

A method is described for the extraction and high-performance liquid chromatographic separation and quantitation of naturally occurring tocopherols and tocotrienols in feedstuffs. Validation of the method in feedstuffs is also reported including reproducibility, linearity, recovery, and precision. A survey of U.S. feedstuffs was performed by using this developed method, and the results are presented showing the α-, β-, γ-, and δ-tocopherols and the α- and γ-tocotrienols of animal feedstuffs collected in five major areas of the United States. Assay results from 77 samples are included.

There are eight naturally occurring forms of vitamin E: α-, β-, γ-, and δ-tocopherol and α-, β-, γ-, and δ-tocotrienol. Various analytical techniques have been used to detect, separate, and quantitate these compounds. Bunnell et al. (1968) reported a comprehensive survey of the α-tocopherol content of feedstuffs in which gas–liquid chromatography was used for the determination of α-tocopherol in alfalfa and secondary magnesium phosphate chromatography followed by two-dimensional thin-layer chromatography used for the separation and determination of α-tocopherol and α-tocotrienol. Bieri et al. (1970) and Ames (1971) reported the use of colorimetric methods to determine α-tocopherol and total tocopherols. Other significant contributors to vitamin E analytical methodology include Lovelady (1973), Tangney et al. (1978), and Slover (1971). Bunnell (1971) provides a thorough review of the development of analytical procedures for vitamin E.

The advent of high-performance liquid chromatography (HPLC) as an accepted technique for vitamin analysis has provided the analytical chemist with methods that offer advantages in speed, accuracy, and specificity over those available in the past. Abe et al. (1975), Vatassery et al. (1978), and Carpenter (1979) have reported the HPLC separation of α-, β-, γ-, and δ-tocopherol. Thompson and Hatina (1979) used HPLC to separate and determine the four tocopherols and α-, β-, and γ-tocotrienol in a variety of tissues and foods. Manz and Philipp (1981) reported a method for the determination of α-tocopherol and α-tocotrienol in animal feeds and human foodstuffs. Other applications of HPLC in vitamin E analysis include the work of Eriksen (1980), Cohen and Lapointe (1980), and Widicus and Kirk (1979).

The purpose of this investigation was to determine the tocopherol and tocotrienol contents of a wide variety of feedstuffs by using HPLC. The separation and identification of all eight vitamin E isomers were achieved. However, the quantitation of β- and δ-tocotrienol was not reported due to the instability and insufficient purity of available standards. The procedure has proved itself to be reliable and suitable for routine laboratory use including the determination of the α-tocopheryl acetate content of mixed feeds.

MATERIALS AND METHODS

High-Performance Liquid Chromatography. The HPLC system used consisted of a Model 950 HPLC pump (Tracor Inc.), a Model 650-10 LC fluorescence detector (Perkin Elmer Corp.) with excitation at 294 ± 2 nm and emission at 325 ± 2 nm, a Model 7120 injector (Rheodyne) equipped with a 20-μL loop, and a normal-phase Chromegasphere SI 60, 5 μm, column, 15 cm × 4.6 mm i.d. (E. S. Industries). Chromatograms were recorded and peak areas determined by using a Model 3390 integrator (Hewlett-Packard). The mobile phase was 2.5% (v/v) tetrahydrofuran in isooctane with a flow rate of 1.5 mL/min. The injection volume was 20 μL. All solvents used

Hoffmann-La Roche, Inc., Product Development & Applications, Nutley, New Jersey 07110.

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